APPENDIX D: BIOPSY SAMPLING PROTOCOLS

COLLECTING TISSUE WITHOUT REFRIGERATION FOR GENETIC ANALYSIS

MATERIALS FOR COLLECTING GENETIC TISSUE SAMPLES

* scotch tape to protect writing on the vials
* pencil to write on label
* waterproof label, 1/4" x 4"
* permanent marker to label the vials
* screw-cap vial of saturated NaCl with 20% DMSO*, wrapped in parafilm
* piece of parafilm to wrap the cap of the vial after sample is taken
* latex gloves
* plastic board, ~6" x 4"
* Betadine swabs
*alcohol swabs
* 4 - 6 mm biopsy punch - sterile, disposable, for boated turtles
* vial with sterile stainless corer for turtles not boated
* whirl-pak to return / store sample vial

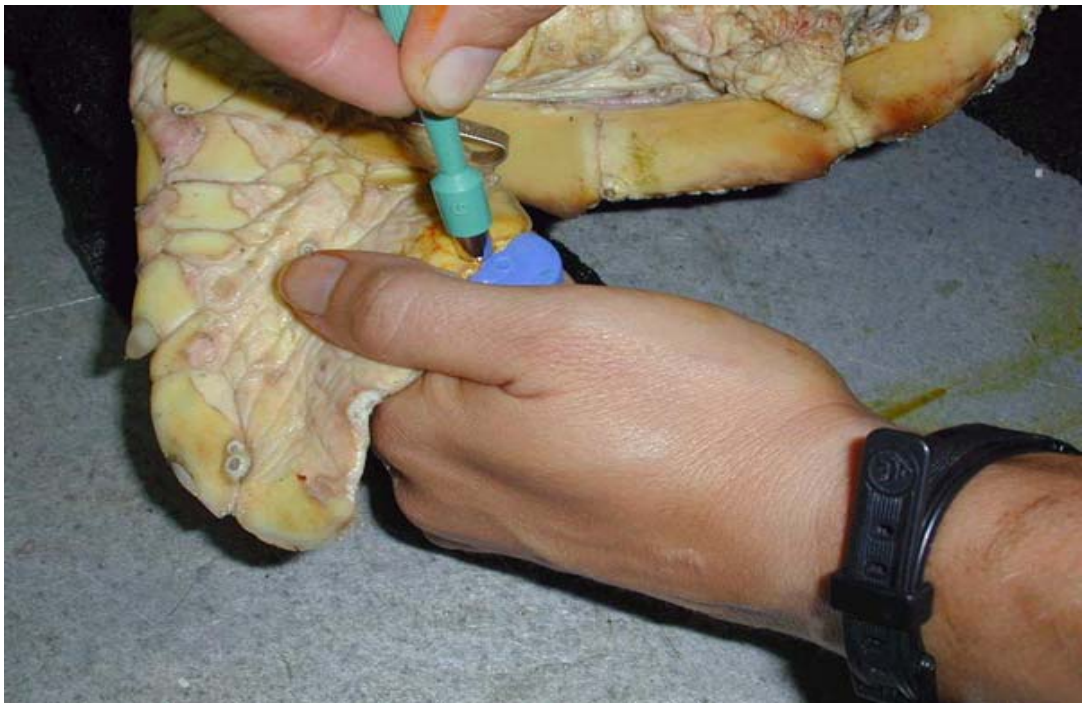
We have included two types of biopsy kits in each sampling case: one for turtles not boated and one for turtles boated. The one for turtles not boated can be distinguished by the presence of two types of vials: one for the storage of the dry, sterile corer and one that contains a preservative into which the corer is placed once a sample is taken. The kits for turtles that are boated contain but one type of vial and also contain sterile individual wrapped biopsy punches.

Protocol for Live Turtles that are Boated

1. Turn the turtle over on its back.
2. Swab either the plastic board or the cap of the vial with alcohol.
3. Wipe the ventral surface of the rear flipper 5-10 cm from the posterior edge with the Betadine swab. Note: leatherbacks cannot be turned over and the sampling location is opportunistic, ideally in the trailing edge of one of the flippers.
4. Place the cleaned plastic board underneath the Betadine treated rear flipper. OR use the cleaned vial cap as the hard surface under the rear flipper.
5. Press the new biopsy punch firmly into the flesh as close to the posterior edge as possible and rotate one complete turn. Cut all the way through the flipper to the plastic board or cap. Use a new biopsy tool when collecting from each different animal to prevent cross-contamination. Also be sure to clean board or cap first with alcohol.
6. Repeat the tissue punch process twice with the same punch, to end up with 2 plugs from the one animal.
7. Wipe the punched area with Betadine.
8. Push out the tissue plugs into the vial containing saturated NaCl with 20% DMSO. Also, you can tap the edge of the punch against the inside of the vial.

9. Use the pencil to write the trip number, specimen number, species id, and carapace length (SCL_{n-t}) on the waterproof paper label and place it in the vial.
10. Label the outside of the vial using the permanent marker with trip number, specimen number, species id, and carapace length (SCL_{n-t}).
11. Apply a piece of clear scotch tape over what you have written on the vial to protect the writing from being erased or smeared by an accidental spill of ethanol.
12. Wrap parafilm around the cap of the vial by stretching it as you wrap.
13. Place vial within whirlpak and close.
14. If using the plastic board, wipe it clean with alcohol swab.
15. Dispose of the used biopsy punch in the sharps container and all trash in a trash bag/container.
16. Submit the vial with your datasheets. Be sure to indicate on your datasheet that a biopsy sample was taken.

Questions: Sea Turtle Program NOAA - NMFS / SEFSC 75 Virginia Beach Drive, Miami, Florida 33149. (305) 361 - 4207.



Flipper biopsy technique (photo by J.B. McNeill).

The 20% DMSO buffer within the vials is nontoxic and nonflammable. Handling the buffer without gloves may result in exposure to DMSO. This substance soaks into skin very rapidly and is commonly used to alleviate muscle aches. DMSO will produce a garlic/oyster taste in the mouth along with breath odor. The protocol requires that you wear gloves each time you collect a sample and handle the buffer vials. DO NOT store the buffer where it will experience extreme heat. The buffer must be stored at room temperature or cooler, such as in a refrigerator.

Protocol for Turtles Not Boated

The sampling gear consists of a 4' anodized aluminum biopsy pole section and a 4' handle section with a 4' extension; these are manufactured by ARC (Aquatic Release Conservation). Assemble the sections together to attain the desired length. The corer is attached to the end of the biopsy pole section.

The corers have been sterilized and are stored in ethanol-cleaned vials. When a turtle that cannot be boated is alongside the vessel, the corer should be attached to the biopsy pole section. Clean the end of the threaded stud on the biopsy pole section with an alcohol swab. Carefully remove the corer from its vial and screw it tightly on the end of the stud.

Observers have been successful in obtaining samples in a number of ways. Some have employed a forceful jab perpendicular to the flipper while others were more successful using an oblique angle. Others "scraped" tissue with the corer. Considerable force may be needed to penetrate the skin of some turtles. Suitable sampling sites include anywhere on the flippers, shoulders, and pectoral and pelvic regions. (High on the shoulders, near the carapace are nerve bundles that should be avoided as should the armpit area which is heavily vascularized.) Do not target the carapace with a jabbing motion, but if this sampling occurs, the depth of the corers (1 cm) is such that no permanent damage will result. Some observers have been able to get samples by "scraping" the leatherback's carapace filling the corer. Scraping leaves a gray superficial scar that should heal fine over time.

Due care should be taken not to strike a crew member when swinging the pole aboard – the corers are sharp. The corer should be removed once the pole is brought back on deck. Once unscrewed, the entire corer with tissue sample inside should be placed into the salt saturated DMSO vial of preservative provided. No attempt should be made to remove the tissue from the corer. Clean the adapter stud with an alcohol swab and store your equipment. The vial should be labeled per the same protocols as for the biopsies of boated turtles.